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Original Research Article

Relationship between *Proteus mirabilis ureC1* Genes in Patients with Urinary Tract Infections [UTIs] and Bladder Cancer

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Abstract

Urinary tract infections [UTIs] are more frequently linked to Proteus mirabilis. To isolate P. mirabilis, 100 urine samples were taken from patients at the City of Medicine Teaching Hospital in Baghdad City who had recurrent UTIs. and look into the ureC gene's distribution. The purpose of the current study was to examine the ureC genes in Gram-negative bacterial isolates from UTI patients and how they differed among bladder cancer patients. Patients with urinary tract infections [UTIs] provided one hundred urine samples. Fifty urine samples from patients with bladder cancer were included in the first group, while fifty urine samples from patients without the disease were included in the second group. The age range of the groups was 15 to more than 65. Using biochemical testing, the gram-negative bacterial species responsible for UTIs were examined and identified. Gender, age groups, and bladder cancer were among the variables linked to UTIs that were investigated. The P. mirabilis isolates were identified based on the morphological and biochemical features of the cultures, and the diagnosis was confirmed using the VITEK 2 compact system. The polymerase chain reaction [PCR] method using ureC genes was used for final identification. The distribution of P. mirabilis by age was significant, with a higher distribution in the 50–55 age range. IL-6: control 13.2+2.8 pg/ml, patients with urinary tract infections [UTIs] and with bladder cancer 12+1.9, patients with urinary tract infections [UTIs] with non-bladder cancer 13.5+4.1. IL-8 levels were: control 1542+123.2 pg/ml, patients with urinary tract infections [UTIs] and with bladder cancer 2542.8+265.8, patients with urinary tract infections [UTIs] with non-bladder cancer 3212+231.2

Keywords: *ureC* gene, IL 8, IL 6, Urinary Tract Infections, bladder cancer.

INTRODUCTION

Proteus mirabilis is more commonly associated with urinary tract infections [UTIs]. It is the third most common cause of severe UTI, after Escherichia coli and Klebsiella pneumoniae, and the second most common cause of catheter-associated bacteriuria, after Providencia stuartii, in long-term catheterized patients [1].

The breakdown of urea by urease, the urease enzyme that causes kidney and bladder stones, the ure operon that produces the urease enzyme, and ureC, a key gene that drives urease synthesis, are just a few of the potential virulence factors that could contribute to the pathogenesis of P. mirabilis [2]. The MR/P fimbriae of P. mirabilis have been studied the most. The genes encoding MR/P fimbriae [mrpA] constitute a complicated fimbrial operon. Several of these genes have been mutated in P. mirabilis, and these mutants have been employed in infection studies to show how MR/P fimbriae contribute to the pathogenesis of P. mirabilis UTIs [3]. Urease, which either clogs the urinary tract or catalyzes the formation of kidney and bladder stones, is involved in the pathophysiology of Proteus mirabilis. Urease has a major role in Proteus mirabilis pathogenicity. This enzyme causes kidney and bladder stones to form or encrusts or obstructs the urinary tract [4]. The cluster of urease genes [ureRDABCEFG] encodes the multimeric nickelmetalloenzyme that hydrolyzes urea into ammonia and dioxide, increases PH, and induces multifunctional urinary ion precipitation that leads to stone formation. Cancer is caused by abnormal cell formation that can infiltrate and spread to other body parts [5]. Despite significant improvements in treatment over the past few decades, cancer remains the second most prevalent cause of death globally [6]. Urinary tract infections, which can be caused by prolonged immunosuppression, complex

cancer treatments, or the use of urinary catheters for patients with urinary system malignancies, including bladder cancer, are among the most common infections in cancer patients [7]. One of the most significant problems UTI patients face is the development of antibiotic resistance in gut flora. As a result, researchers have created new antibiotics to combat resistant bacterial strains [8]. These cytokines induce decidua and chorionic membrane cells to release IL-6, which facilitates membrane rupture, in conjunction with IL-8. Elevated levels of IL-1b and IL-8 in the lower uterine tract during labor have been associated with cervical dilatation [9]. Meanwhile, studies have shown that under normal conditions, the cervix fibroblast is responsible for IL-8 synthesis [10]. However, because it starts and maintains the immune response against the aggressor agent, the production of cytokines by immunocompetent cells—such as lymphocytes, macrophages, natural killer cells, and others—is essential to the infection process [11].

Objectives of the study:

1. Isolation of Proteus mirabilis from UTI-causing bacteria in cancer and non-cancer patients
2. P. mirabilis UTI isolation resistance genes in cancer and non-cancer patients
3. Measure the levels of IL-6 and IL-8 in cancer patients, non-cancer patients, and healthy people who have bacterial UTIs.

MATERIAL AND METHODS

Collection of Samples

Between November 2025 and March 2026, urinary tract infection [UTI] patients who visited the Baghdad Teaching Hospital, City of Medicine, provided samples. The age range of the groups was 15 to over 65. 50 urine samples from patients with UTIs who did not have bladder cancer, 50 urine samples from patients with bladder cancer, and 25 urine samples from control groups made up the total of 100 samples. This holds true for both men and women. Patients' information was gathered by creating a thorough questionnaire.

Urine Sample Collection

As mentioned in patients were instructed to gather midstream urine. After that, a flame-sterilized culture loop was used to cultivate the samples. After that, differential media [Maconkey agar, Xylose lysine deoxychoclate agar and blood agar] were incubated under aerobic conditions for 24 hours at 37°C. Following bacterial growth, the colonies were plated onto fresh plates and subcultured to produce pure bacterial colonies. Prior to diagnostic testing, pure isolates were kept at 4°C. The leftover urine samples were put in separate tubes and centrifuged for five minutes at 3500 rpm. Urine test strips with ten tests were used to identify the following components after the morphological features of the filtrate were noted: yellow pigments, ketone bodies, protein, glucose, pH, and density. Red blood cells, pus cells, epithelial cells, bacterial cells, and crystals were among the cells and components found on a clean glass slide that was examined under a microscope at 40x magnification after a drop of the precipitate was placed on it using specific gravity and nitrate.

Isolation of *Proteus* isolates

Blood agar, MacConkey agar, and Xylose lysine deoxychoclate agar [XLD agar] plates were streaked with all samples. XLD agar is a selective and differentiation medium that is used in conjunction with the other media to identify Enterobacteriaceae. For a whole day, the plates were incubated aerobically at 37°C.

Identification of *Proteus mirabilis* isolates

Bacteriological Assay

Gram stain was used to identify the isolates based on their form, organization, and reactivity to the stain. Additionally, morphological characteristics on culture media were analyzed, including colorless growth on [XLD] agar, non-lactose fermented growth on MacConkey agar, and swarming on blood agar.

Biochemical Tests

To identify bacteria growing on culture media after 24 hours of culture and the appearance of growth on the culture medium, a number of biochemical tests [IMVIC, triple sugar iron, urease test, oxidase test, catalase test, motility test, gelatin test] were carried out.

Cytokine determination

Using ICN Biomedical Inc. kits and enzyme-linked immunosorbent assays [ELISA], the IL-6 and IL-8 measurements were performed in accordance with the protocol guidelines. In short, samples and standards were incubated on immunoplates coated with monoclonal antibodies to the investigated cytokine. Following washing, each cytokine-specific biotin-conjugated polyclonal antiserum was added, and the plates were further incubated and cleaned. Tetramethyl bencidine was used to create the color response when avidinperoxidase was introduced, followed by incubation and washing. An ELISA plate reader was used to evaluate absorbencies at 450 nm after SO4H2 was introduced as a stopper. Standard curves based on prepared dilutions of recombinant cytokines were used to compute values.

Vitek 2 Identification System

The VITEK 2 method [BioMe'rieux] is a unique automatic technique that uses fluorescence-based technologies to identify bacteria and selection tests.

Molecular Methods

Polymerase Chain Reaction

After a number of experiments, the ideal conditions for [Initial denaturation and annealing] were found. The temperature was adjusted using gradient PCR for all samples in order to choose the ideal condition, and

the concentration of DNA template was adjusted between 1.5 and 2 µl. These two factors were taken into consideration from significant factors in primer annealing with complement.

DNA Extraction

All 100 Proteus mirabilis clinical isolates had their genomic DNA extracted using the ABIOpure Extraction technique. DNA, Concentration, and Purity Estimation Using Nanodrop, the concentration of the isolated DNA was measured in 30-50 ng/µm and the purity was determined by observing the ratio of O.D. 260/280. One microliter of the extracted DNA was added to the device. Using 1% agarose gel electrophoresis, the acceptable 260/280 ratio for pure DNA is between 1.7 and 2.

Primers

The primers used in the interaction

IDT [Integrated DNA Technologies company, Canada] examined the primers after they were lyophilized, dissolved in free ddH2O to yield a final concentration of 100 pmol/µl as stock solution, and kept a stock at -20 to prepare a 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume of 100 µl.The interaction's primers,.

Table 1: The specific primer for ure*C1* gene

Primer	Sequence	T_m [°C]	GC [%]	Product size
Forward	5'- CCGGTTCAGAAGTTGTGGCTG GA- 3'	79.3	56.0	533 base pair
Reverse	5'-GCGCTCTCCTACGGACTTGATT - 3’	68.4	59.1	

Analysis of statistics

The ANOVA test was used to assess multiple comparisons between the experimental categories and the control. The data is expressed using mean±SE and other descriptive statistics.

RESULTS

Sample Distribution

According to Bacterial Growth

50 urine samples from bladder cancer patients with UTIs and 50 urine samples from people without cancer with UTIs were collected. Table [2] indicates that 40 [80%] of the patients with UTIs who had bladder cancers had positive bacterial growth in various culture media, whereas 35 [70%] of the patients with UTIs who did not have bladder cancers had positive bacterial growth in various culture media, and 25 urine samples from the control groups had nonbacterial growth.

Table 2: Patients with UTIs distributed based on bacterial growth

Samples	[+] growth		[-] growth		Total	
	No.	%	No.	%	No.	%
bladder cancers	40	80	10	20	50	100
Without cancer	35	70	15	30	50	100
Control groups	0	0	25	100	25	100

Estimation of immunological markers [IL 1 β, IL 8] in bladder cancers and non-cancer patients infected with urinary tract infections and control.

The interleukin IL 6 pg/ml and IL 8 level [P<0.01] in various types of bladder cancer patients with UTIs were not significantly different from those of non-cancer patients with UTIs. Table 3 presents these results.

Table 3: Interleukin 6 and IL 8 levels in patients with bladder cancer and those without cancer who have urinary tract infections and control

Immunological markers	Mean + Std. Deviation		
	Bladder cancer	Without cancer	Control
IL 6 pg/ml	12+1.9	13.5+4.1	13.2+2.8
IL 8 pg/ml	1542+123.2	2542.8+265.8	3212+231.2
P value	**0.01**	**0.01**	**0.07**

Relationship between patients with bladder cancer and those without cancer who have UTIs and control with age groups.

The letters [A, B, C and D] represented the levels of significance, highly significant starting from the letter [A] and decreasing with the last one.

According to these findings, the majority of bladder cancer patients with UTIs were between the ages of 51 and 60, with a percentage of 15 [30%]. According to Table [4], the larger age group of UTIs without malignancy was 61–70 years old, with a proportion of 15 [30%].

Table 4: Patient distribution based on age

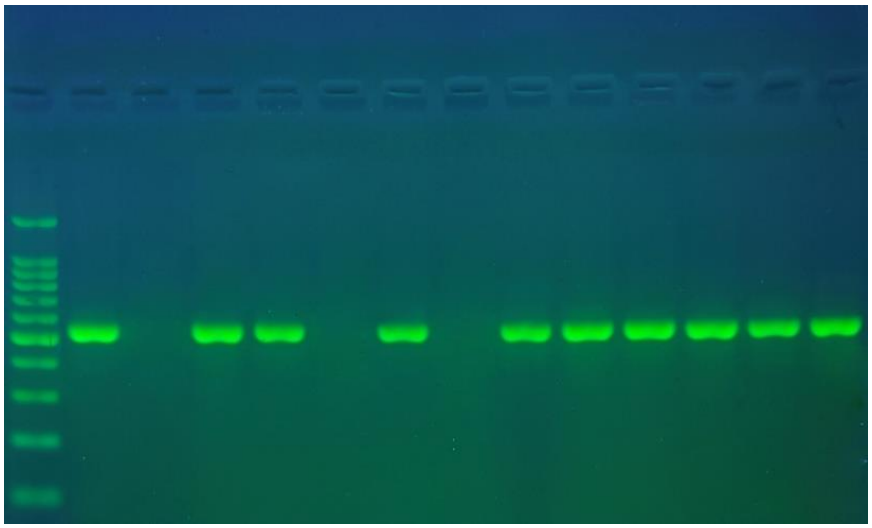
Age group	Bladder Cancer	UTI without cancer	Control
20-30	20[10%]	10[5%]	20[5%]
31-40	10[5%]	16[8%]	8[2%]
41-50	16 [8%]	20[10%]	20[5%]
51-60	15[30%]	12[24%]	32[8%]
61-70	12[24%]	15[30%]	20[5%]
Total no. [%]	50[60%]	50[100%]	25[100%]

Molecular Detection of Virulence Genes

Detection of *ureC* gene

The ureC gene was found in P.mirabilis isolates, the bacterial DNA was amplified for this gene using the PCR technique in a monoplex pattern utilizing certain primers, and the ideal conditions for this gene's PCR amplification were determined.

According to the current study's findings, 11 [78.5%] of the Proteus mirabilis isolates produced positive results at 533 bp, as seen in Figure 1. Agarose gel electrophoresis and ultraviolet [UV] transilluminator photography were used to confirm the ureC gene.



The band size of the PCR result in Figure 1 is 533 bp. Electrophoresis on 1.5% agarose was the end result. TBE buffer once every 1.30 hours. N: DNA ladder [100].

DISCUSSION

The toxic and inhibitory effects of radiation and chemotherapy, which are used to treat bladder cancers, significantly reduce bacterial growth in cancer patients [12]. In patients without bladder cancers, the development of resistant bacteria and the use of antibiotics prior to sample collection may have contributed to the urinary tract infection, which may have been caused by an organism other than bacteria [13]. These findings of positive bacterial growth for individuals with bladder cancer concurred with those of Aldbagh & Al-Hadithi. [14] who found that urine samples from individuals with bladder cancer had positive bacterial growth rates of 45% and 35%, respectively.

According to Hayat et al.'s investigation, the ureC1 gene had the greatest frequency [100%] among all P. mirabilis isolates [15]. These findings are consistent with earlier research by Bunyan *et al.,*[16] and Al Obeidi *et al.,*[17], but they differ from the results of the current investigation, which detected the ureC gene in 78.5% of isolates.

The results of the study conducted in Al-Diwanya City show that the ureC gene, which produces the urease enzyme, is thought to be a diagnostic characteristic of the P.mirablis bacteria. revealed that the ureC gene is present in 29 out of 30 isolates, or 96.66% of them, according to research done using the Multiplex PCR technique [18]. According to Hamdani *et al.,* in Baghdad City, molecular techniques utilizing PCR technique targeting the UreC gene revealed that UreC was present in 90% of the samples [19]

Since cytokines, cytokine receptors, and soluble mediators are frequently engaged in the pathophysiology of various tumor, viral, or inflammatory disorders, significant progress has already been made in understanding their involvement [20]. Certain cytokines have the potential to cause premature labor when bladder cancer and UTI coexist. Additionally, some of them can offer helpful data for pathogenesis research [21].

This investigation shown that at least two cytokines, IL-6 and IL-8 in UTI and IL-8 in urine, are significantly elevated in bladder cancer patients with urinary infections [22]. They had distinct patterns, nevertheless. In fact, bacterial UTIs had significantly greater amounts of IL-6, a cytokine that primarily triggers the acute phase response, while urine had higher levels of IL-8. This may be because IL-8 is a quemokine that draws a lot of leukocytes, which is common in bladder cancer patients with UTIs [21]. Additionally, patients with urinary infections had higher amounts of IL-8 in their urine than those with pyuria. Interestingly, a correlation between the absence of preterm delivery and the absence of IL-6 and IL-8 in the serum of both infected and non-infected patients was found in the group included in this study. Future research aiming at comprehending the potential pathogenic implications of the systemic rise of these cytokines in the commencement of labor and the premature rupture of the bladder membranes will be encouraged by these findings [23].

One of the major important risk factors for UTI is age [24]. These findings corroborated those of Fadhil [2012], who discovered that 40% of bladder cancer patients in age categories 60–69 had a UTI, and Sharma *et al.,*[25], who discovered that 40.5% of cancer patients in age groups 27–37 had a UTI. According to researchers AlDabagh and Al-Hadithi [26], the percentage of cancer patients having UTIs in the age ranges of 45–54 and 55–64 was 29.03% and 22.58%, respectively, which is lower than these findings.

CONCLUSION

1. The presence of numerous virulence factors that increase the pathogenicity of P. mirabilis isolates shows that UreC precisely regulates necessary genes.
2. Urine also contains IL-8 and IL-6. As assessment indicators of infection, both cytokines may be helpful.

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